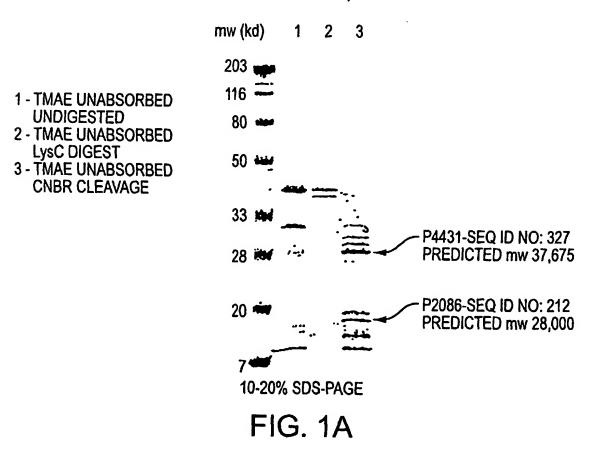
# IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: SDS-PAGE ISOLATION OF PEPTIDES

(CNBr CLEAVAGE OF UNABSORBED TMAE FRACTION FOLLOWED BY SDS-PAGE AND N-TERM SEQUENCING OF FRAGMENTS FROM PVDF BLOT)



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IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: REVERSE PHASE ISOLATION OF PEPTIDES

ENZYMATIC DIGESTION OF UNABSORBED TMAE FRACTION FOLLOWED BY REVERSE PHASE CHROMATOGRAPHY SEPARATION OF PEPTIDES AND DIRECT N-TERMINAL SEQUENCING

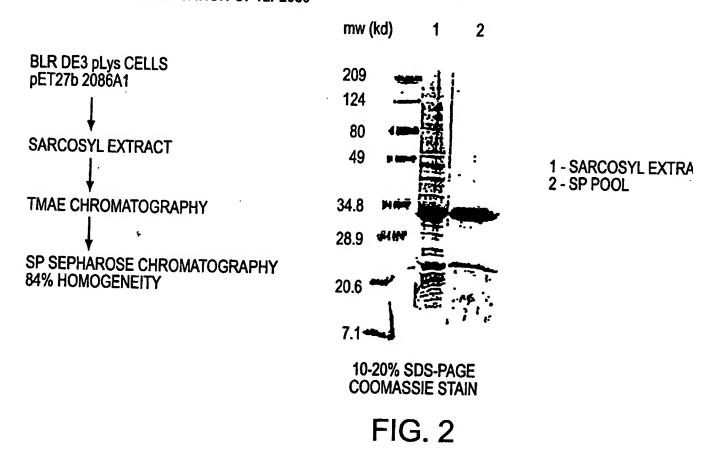
——— <b>≅</b>	<u></u>		(6)	
N-TERM. ID	P5163	P4431	P2086	P5163
MOLECULAR WEIGHT OF PEPTIDE (d)	2069.7	3351.2	3351.2	2278.9
RETENTION TIME OF PEPTIDE (min)	6.716	13.800	13.800	098'9
ENZYMATIC DIGEST	Gluc (V8)	LysC	LysC	ArgC

P4431 (SEQ ID NO: 327) PREDICTED mw 36,775

P2086 (SEQ ID NO: 212) PREDICTED mw 27,100 P5163 (SEQ ID NO: 328) PREDICTED mw 7,081

FIG. 18

### PURIFICATION OF rLP2086

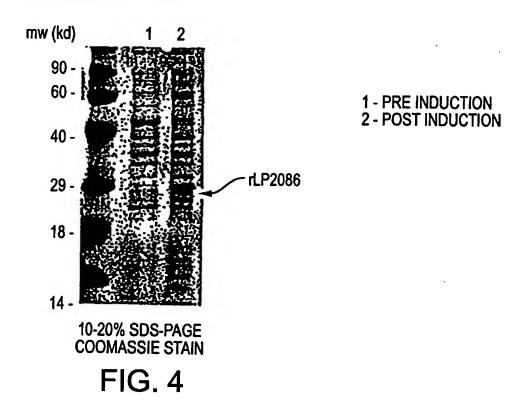


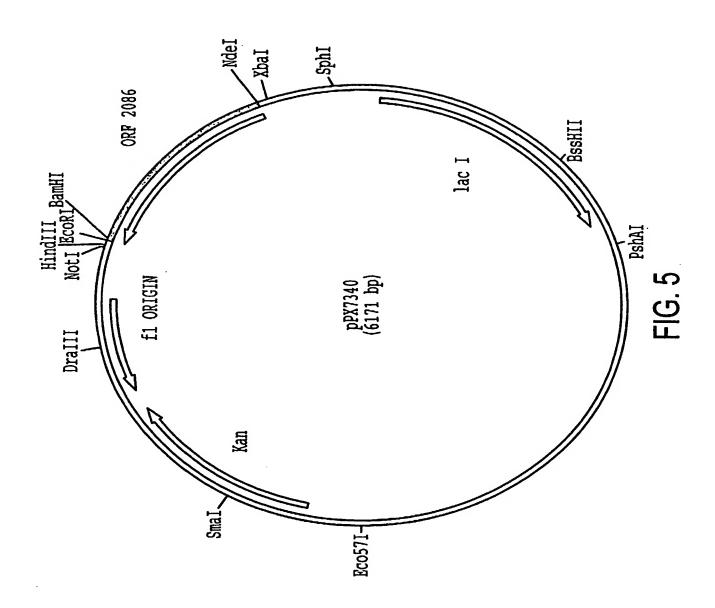
IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: LC-MS/MS

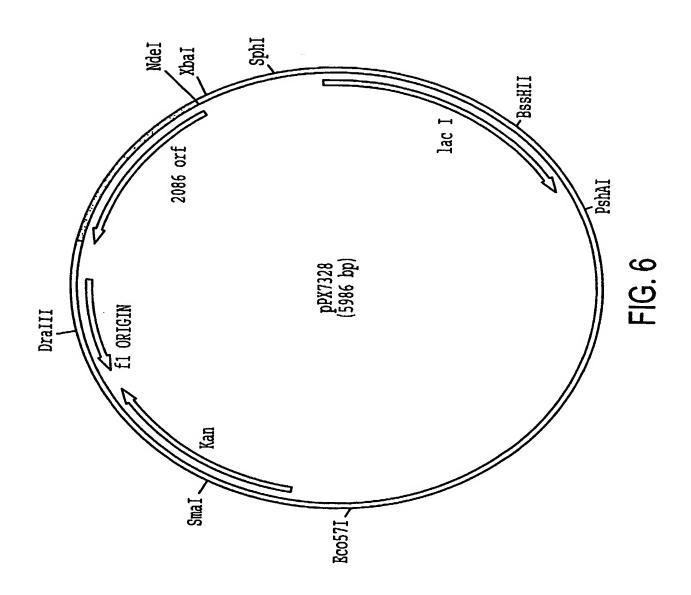
MS/MS ANALYSIS (LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY) SDS-PAGE FOLLOWED BY GEL EXCISION, PROTEOLYTIC DIGESTION, AND LC-



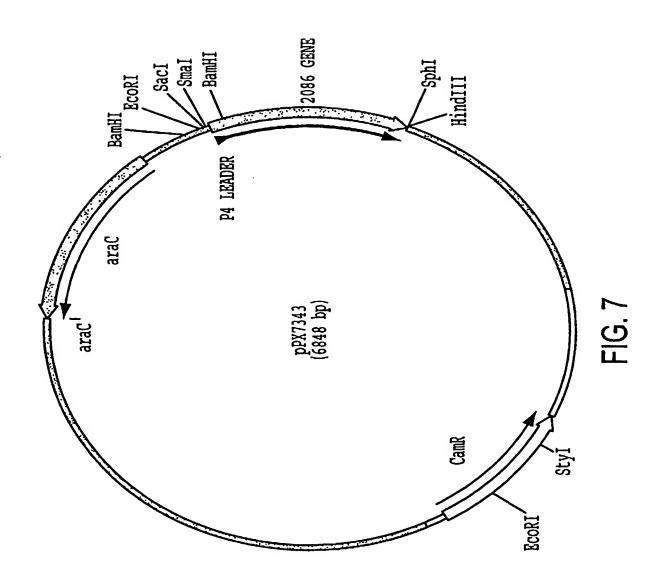
#### **EXPRESSION OF rLP2086**

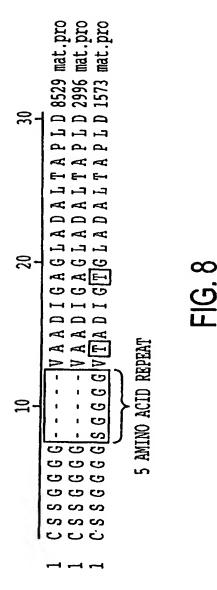






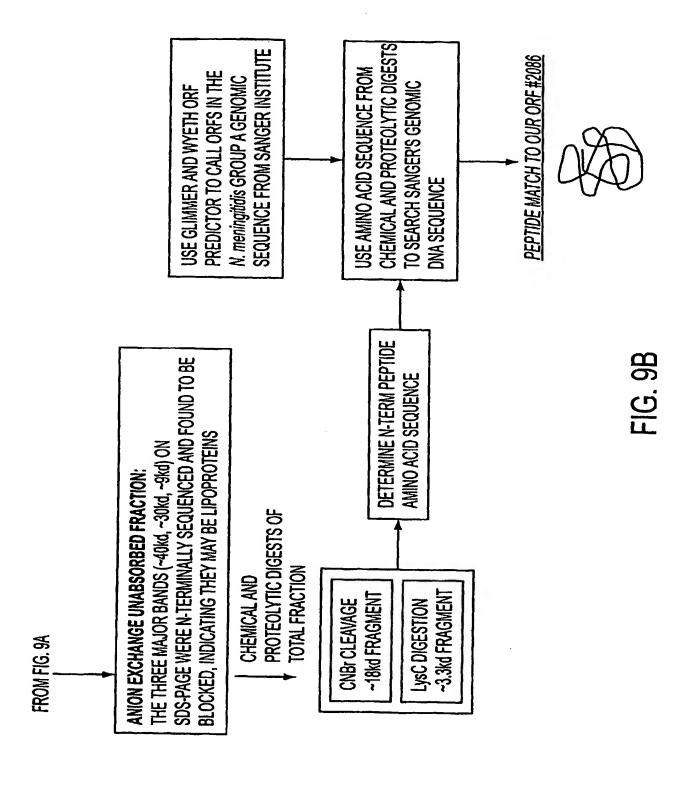
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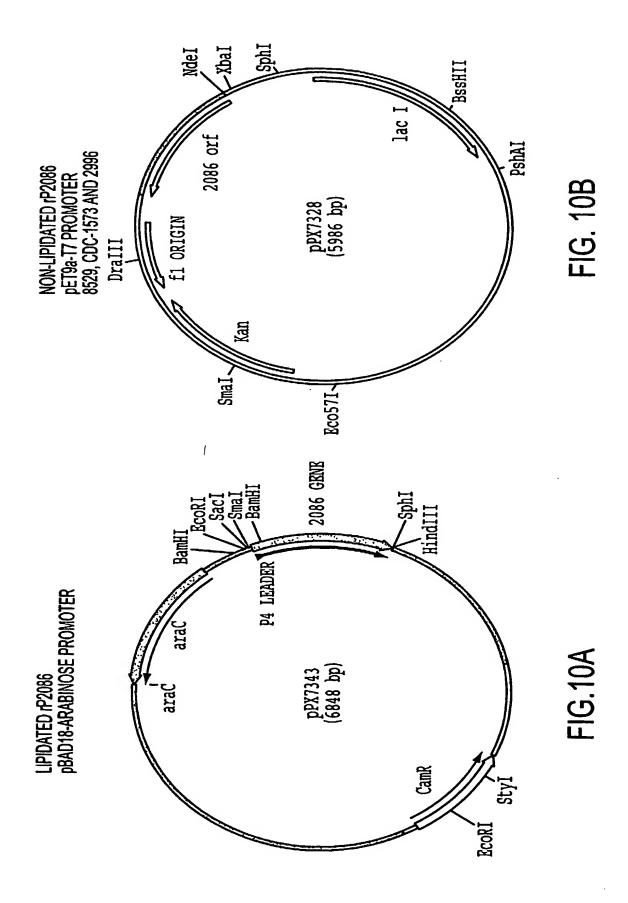


**SUBSTITUTE SHEET (RULE 26)** 

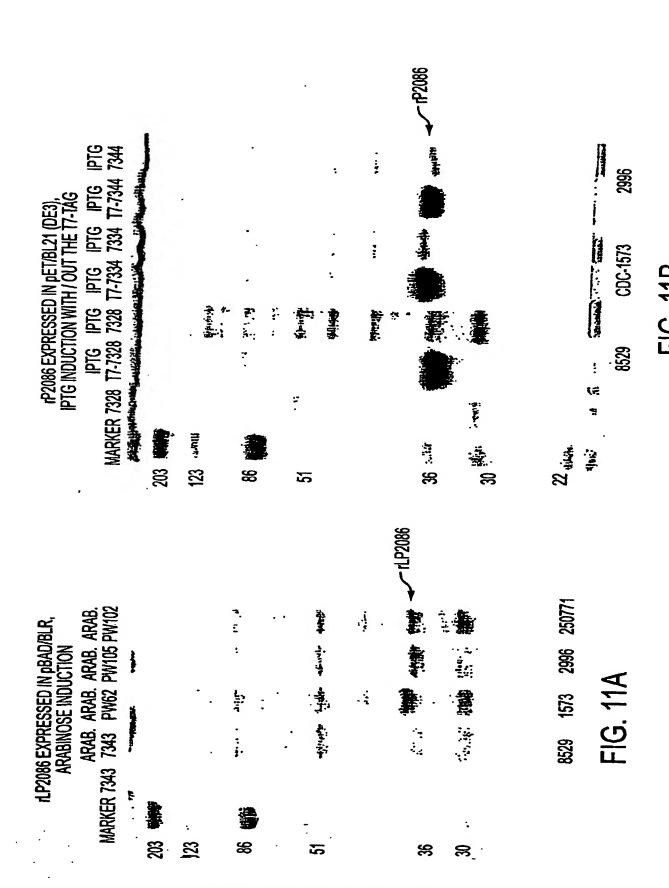
MANUFACTURER'S DIRECTIONS. A 1% 7/10 AND 1% 4/6 AMPHOLYTE SOLUTION WAS USED IN 1% TX-100 PERFORMED IN BioRad Rotofor ACCORDING TO CHROMATOGRAPHY PERFORMED USING TMAE RESIN WITH Tris-HCI pH8 BUFFER AND PROTEIN WAS SEPARATED FROM AMPHOLYTES USING 90% ETHANOL PRECIPITATION COLLECT FRACTIONS IN pI RANGE ~5.6-7.6: B. PREPARATIVE ISOELECTRIC FOCUSING: BC TITERS FOR 4/4 STRAINS TESTED IDENTIFICATION OF IMMUNOGENIC COMPONENT IN Nm STRAIN 8529 UNABSORBED FRACTION: BC TITERS FOR 3/3 STRAINS TESTEL C. ANION EXCHANGE CHROMATOGRAPHY: NaCI ELUTION. 1% TX-100 IN ALL BUFFERS. **TO FIG. 9B** TX-100: BC TITERS FOR 4/5 STRAINS TESTED A. DIFFERENTIAL DETERGENT EXTRACTION ZWITTERGENT 3-14+HEAT **ZWITTERGENT 3-14+NaCI ZWITTERGENT 3-14** ZWITTERGENT 3-12 CYTOPERI SARCOSYL



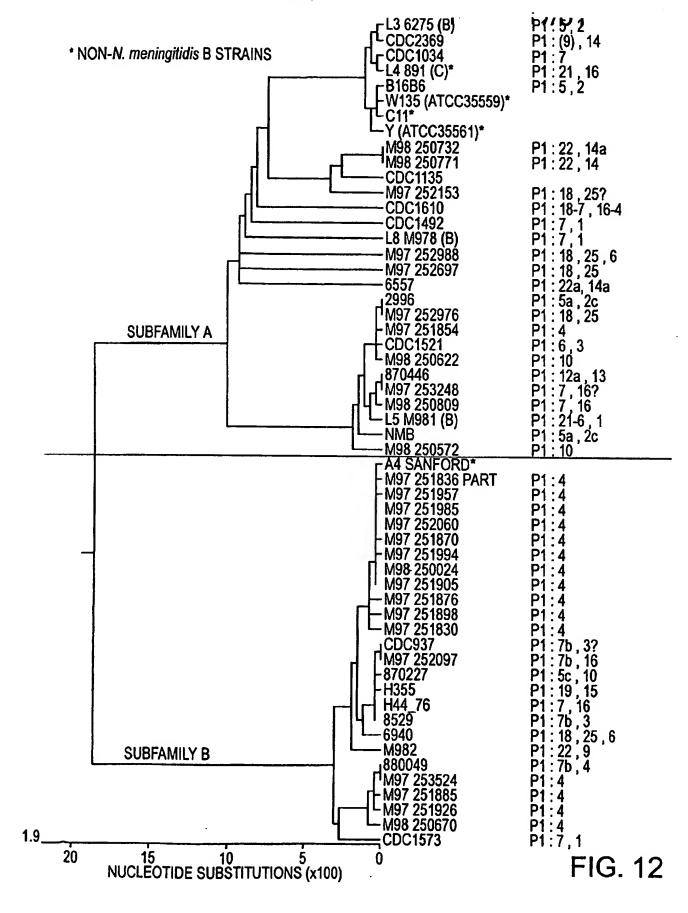
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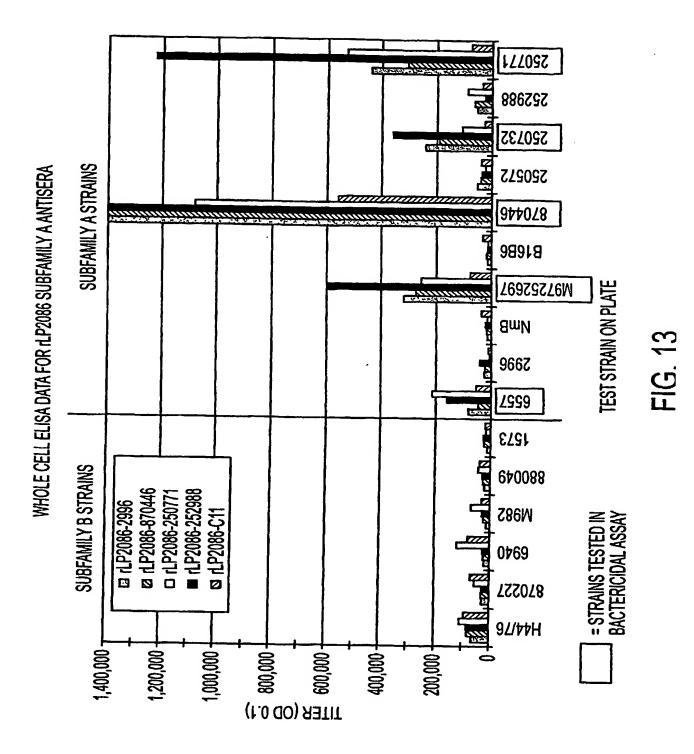


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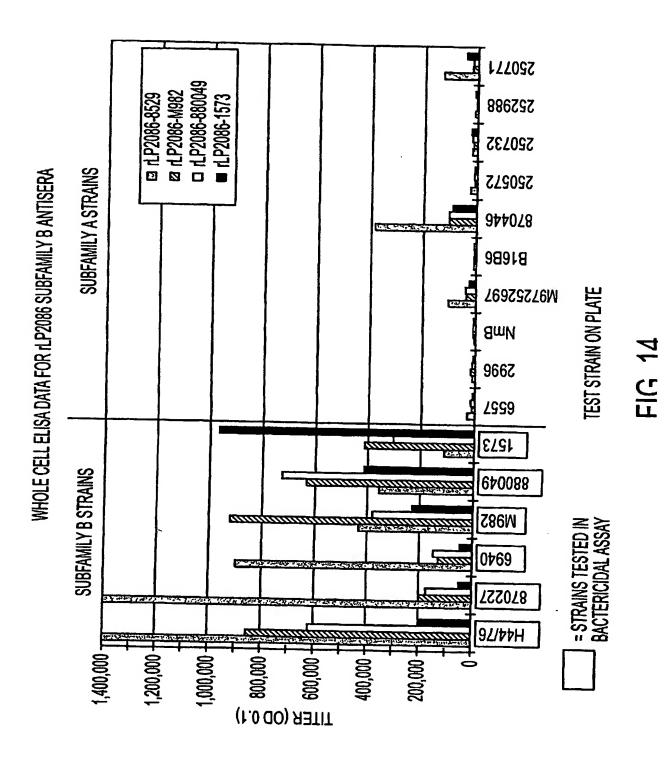


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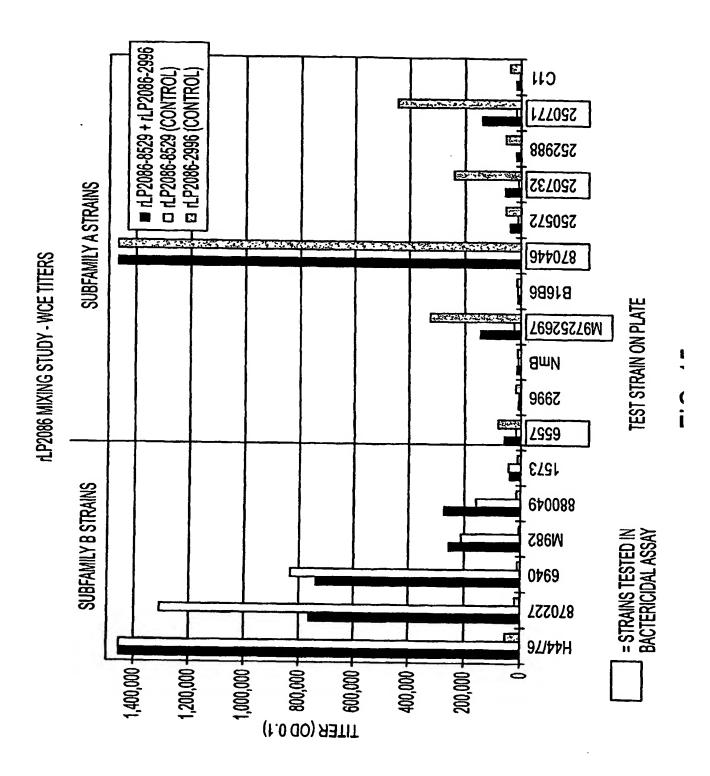


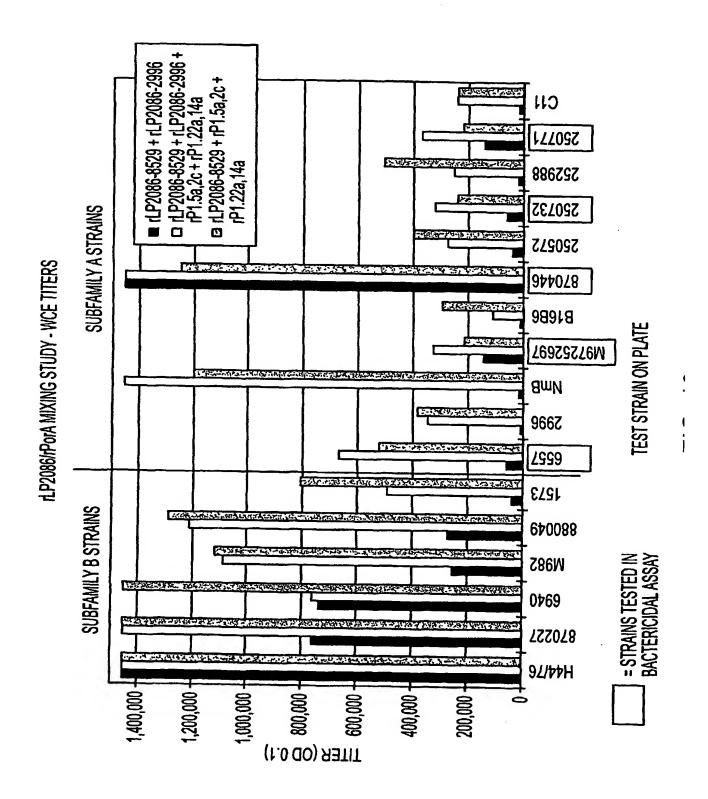


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**SUBSTITUTE SHEET (RULE 26)** 





# WESTERN BLOT REACTIVITY OF rLP2086 MOUSE ANTISERA TO P2086 SUBFAMILY B N. meningitidis WHOLE CELL LYSATES

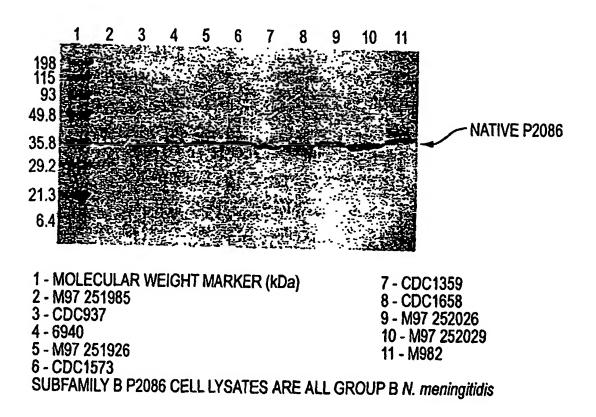
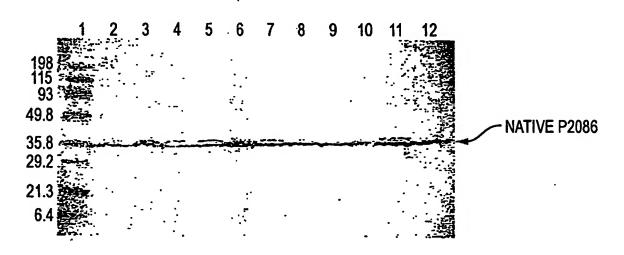


FIG. 17

#### WESTERN BLOT REACTIVITY OF ILP2086 MOUSE ANTISERA TO P2086 SUBFAMILY A N. meningitidis AND N. lactamica WHOLE CELL LYSATES



- 1 MOLECULAR WEIGHT MARKERS (kDa)
- 2 GROUP A N. meningitidis A4 (P2086

SUBFAMILY B)

- 3 GROUP C N. meningitidis C11 4 GROUP Y N. meningitidis -

ATCC35561

5 - GROUP W135 N. meningitidis -

ATCC35559

6 - N. lactamica - UR5

GROUP B N. meningitidis:

7 - CDC1034

8 - M98 250732

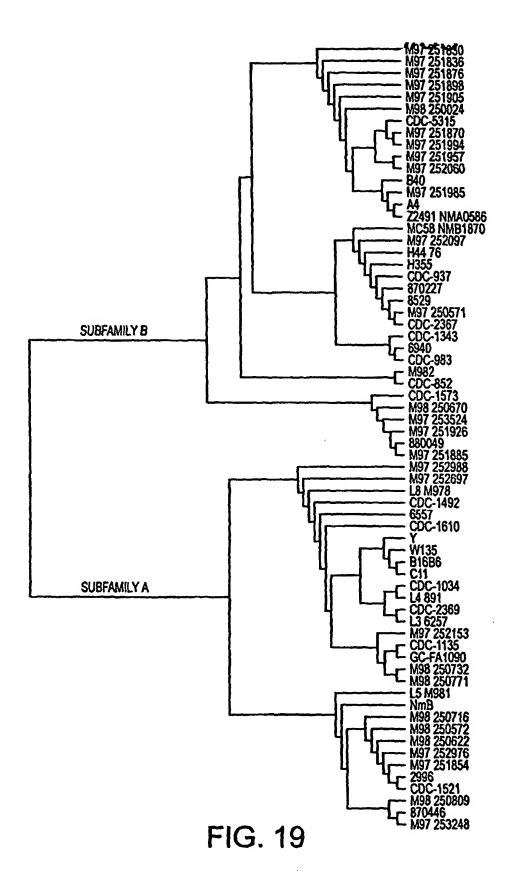
9 - NmB

10 - 6557

11 - CDC1521

12 - M97 252153

FIG. 18



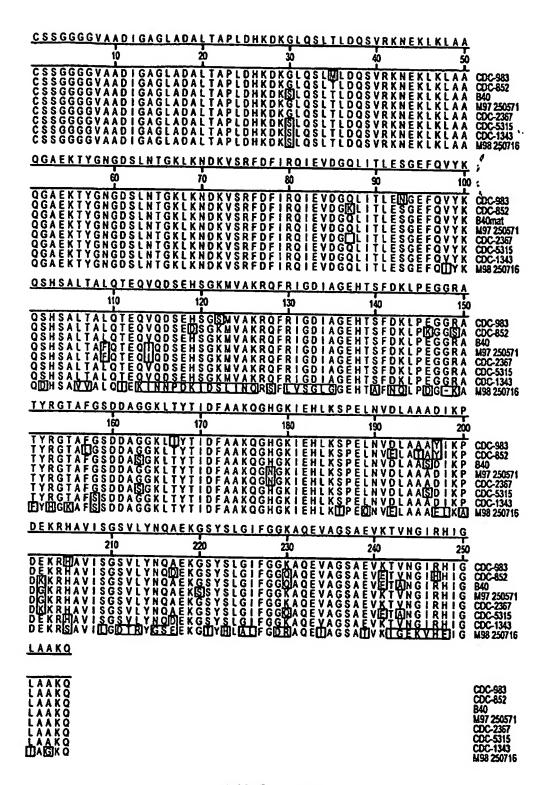


FIG. 20

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